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Development and morphology of *Clathrus delicatus* (*Phallomycetidae*, *Phallaceae*) from India

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Abstract — During fieldwork, *Clathrus delicatus* was collected from the Muthodi forest range in the Bhadra Wildlife Sanctuary in the state of Karnataka, India. Although this species was previously recorded from India, these reports did not include detailed morphological descriptions. Here we describe *C. delicatus* and provide illustrations and notes on fruitbody development, which has not been well characterized in the past.

Key words — *Phallaceae*, peridial suture, primordia, sporoma, volva-gel

Introduction

Members of *Phallales*, commonly called stinkhorns, produce foul-smelling fruitbodies that attract insects. Their distinctive odor is produced by a combination of chemicals such as hydrogen sulfide and methyl mercaptan (List & Freund 1968). Stinkhorns typically develop very quickly, often within few hours, with the spore bearing structures (receptacles) emerging from globose to ovoid structures called 'myco-eggs' (Lloyd 1906, Pegler et al. 1995). The order *Phallales* comprises 2 families, 26 genera, and 88 species (Kirk et al. 2008). Clathroid members of family *Phallaceae* form multipileate receptacles (Gäumann 1952) with beautiful and bright colored sporomata. *Clathrus* is unique in having latticed, hollow, spherical or stellate receptacles with slimy glebae (spore masses) borne on their inner surfaces (Pegler et al. 1995). Species in *Clathrus* have simple (Ingold 1971), ellipsoid spores that are typically dispersed after they adhere to the body parts of insects that have been lured to the fruitbody by its fetid aroma (Alexopoulos et al. 2002).

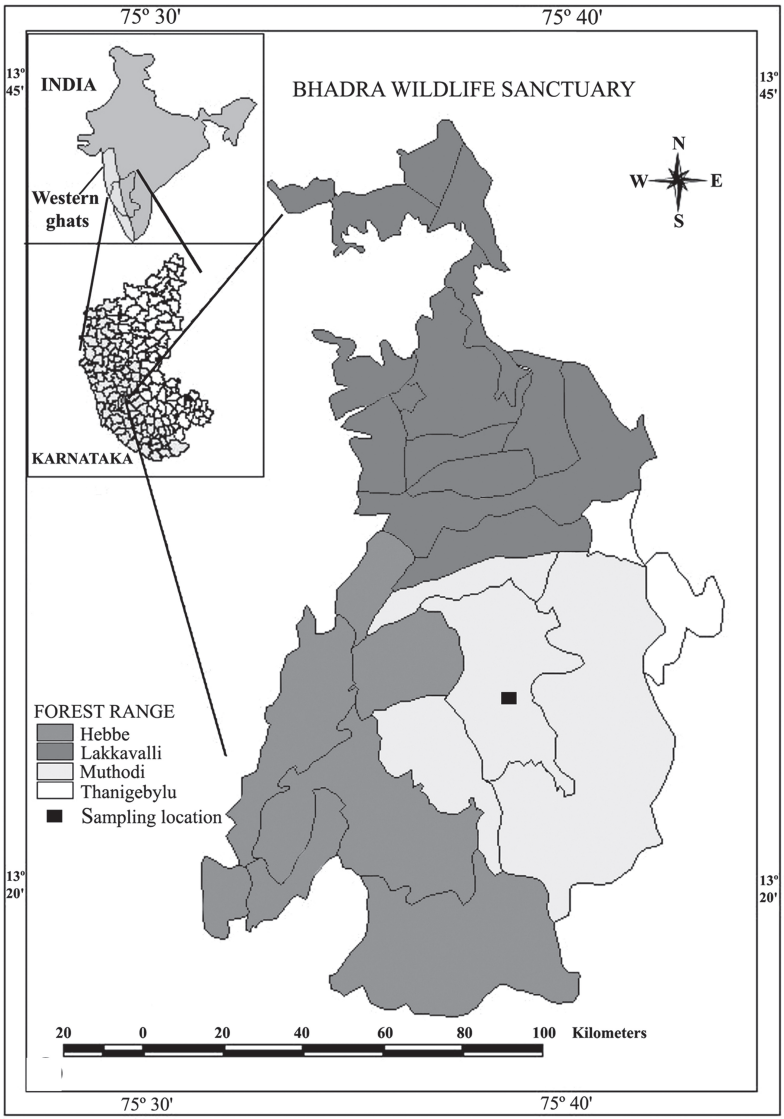


Fig. 1. Sampling location in Muthodi forest range, Bhadra Wildlife Sanctuary.

Clathrus delicatus was first described by Berkeley & Broome (1873). Fischer (1898–99) outlined growth stages of *C. delicatus* and compared its receptacle and glebal development to that of *C. chrysomycelinus* Möller. Narasimhan (1932), who published the first report of *C. delicatus* from India (Mysore, Karnataka),

gave a few details on characteristics of the gleba but did not describe the sporoma morphology (e.g., egg and receptacle color and size). Dring (1980) described the development of sporomata in *Clathraceae* (regarded as synonymous with *Phallaceae* by Kirk et al. 2008) and correlated the relationship of receptacle with the other parts of the developing fruitbody. Later, Apte (2005) collected *C. delicatus* during a survey on Owl moths (*Othreis* spp.) in Sanjay Gandhi National Park, Mumbai and sent the photographs to the Smithsonian Institution (USA) for identification but did not provide a morphological description of *C. delicatus*.

The present paper provides the first detailed taxonomic description of *C. delicatus* based on Indian material collected in India, including a systematic study of the sporoma development of this species.

Materials and methods

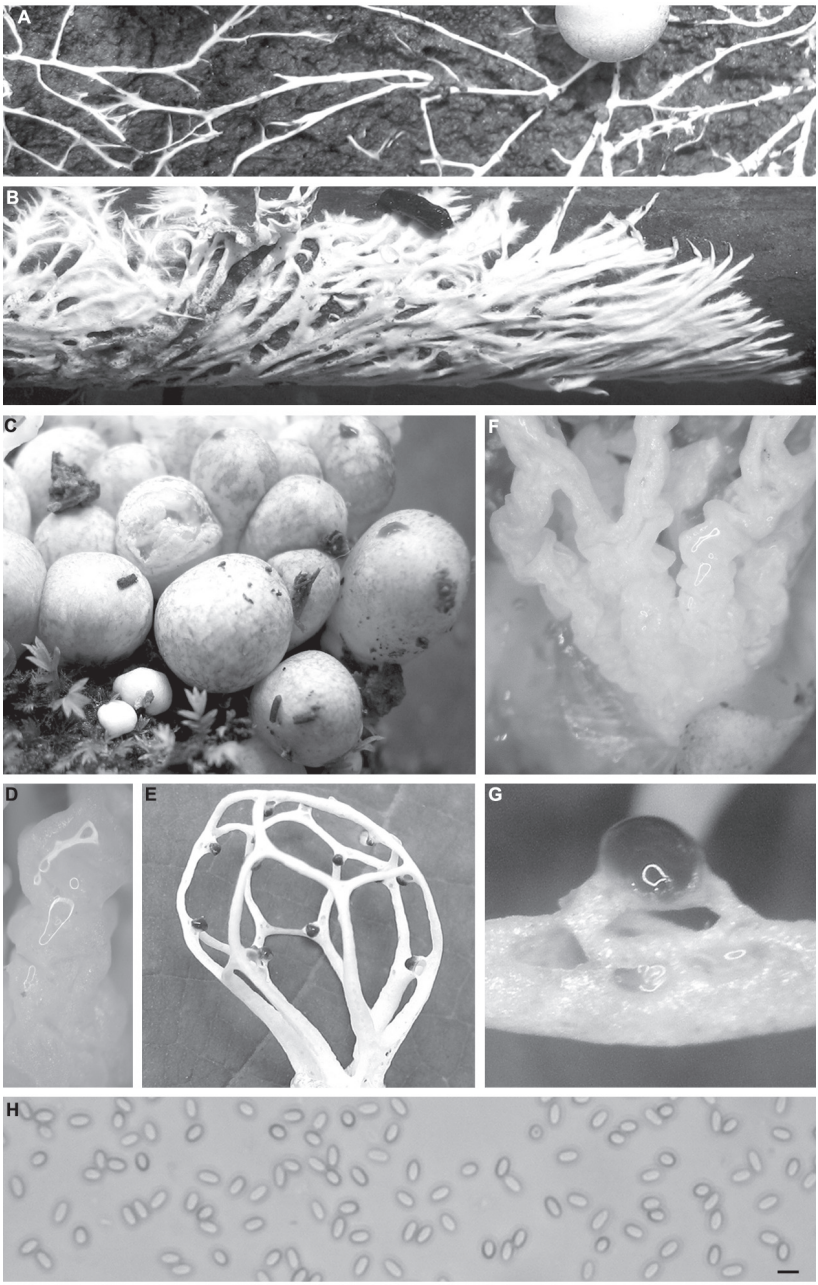
Collections were made at the Muthodi forest range in the Bhadra Wildlife Sanctuary, Karnataka, India (Fig. 1), altitude 700 m, temperature 22–28°C and relative humidity 75–90%. Fresh specimens were photographed and color notations were made according to Kornerup and Wanscher (1978). Descriptions of macroscopical characters were compiled from field notes on fresh specimens. Microscopic observations and measurements were made on mounts of receptacle material in 3% KOH stained with 3% phloxine. The primordia were fixed in Pfeiffer's solution containing methanol (absolute) and 40% formalin (w/v) in equal proportions, and then free-hand sections, stained with 1% lactophenol cotton blue and 1% phloxine, were prepared on glass slides for observations under a stereo microscope. The specimens cited are deposited in the herbarium of the Department of Applied Botany, Kuvempu University, Shankaraghatta, Shimoga Dist., Karnataka, India (KUABSAK).

Taxonomy

Clathrus delicatus Berk. & Broome, J. Linn. Soc., Bot. 14: 77, 1873 ["1875"]

Figs. 2–4

Immature Fruit Bodies ('myco-eggs') arising from thick whitish (1A1) mycelial strands (Fig. 2A) running over twigs (Fig. 2B); globose to ovoid (Fig. 2C), white (1A1) to pale orange (5A1-3), up to 10 mm in diameter, rupturing apically to reveal the expanding receptacle that is initially covered in a mucilaginous substance (Fig. 2D). Receptacle hollow with latticed network, 15–20 × 10–14 mm (Fig. 2E), chalk white (1A1), meshes about 10–12, polygonal, irregularly branched, ± isodiametric towards the apex and vertically elongated towards the base, where arms unite to form a short stipe (Fig. 2F). Arms smooth, flattened, each deeply grooved along their outer-surface. Gleba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olive brown (4E6), initially coralloid, mucilaginous, deliquescing jelly-like masses restricted to the inner surfaces of the receptacle (toward the apex where arms intersect) on specialized organs (resembling three-legged stools) called glebifers (Fig. 2G). Volva pale white to light orange (5A4), thin, enclosing the basal portion of the receptacle. Basidiospores elliptical, 1–2.2 × 3.6–4.8 µm, smooth, hyaline (Fig. 2H).

Specimen examined: INDIA, Karnataka, Muthodi Forest Range, Bhadra Wildlife Sanctuary (13° 21' 13" N, 75° 38' 10" E, alt. 700m), on decaying vegetation of *Bambusa arundinacea* Retz. (*Poaceae*), 20.VIII.2007, coll. S. Swapna, S. Abrar, C. Manoharachary & M. Krishnappa (KUABSAK-MCH265).

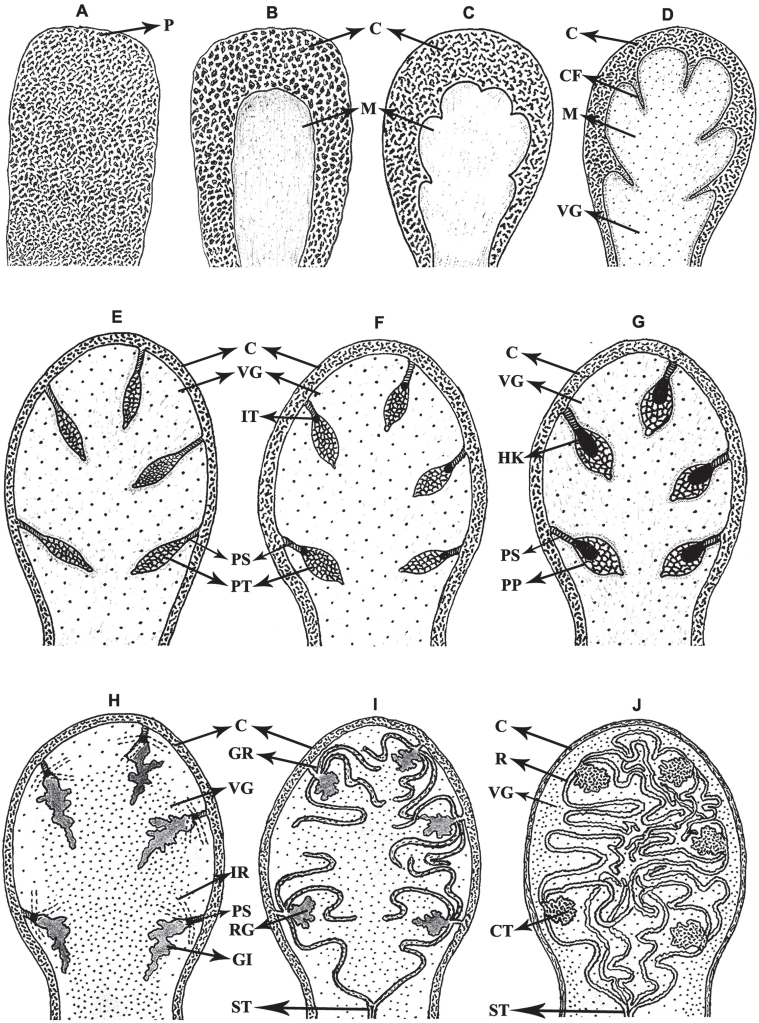
Development

C. delicatus undergoes two phases in the sporomic stage: a myco-egg phase and receptacle phase.

Primordia Initiation: Primordia initiate at points of swellings along the mycelium strands. The primordium initial (P) lacks an internal structure and is composed of hyphal elements (Fig. 3A). The developing primordium differentiates into central medulla (M) and peripheral cortex (C) (Fig. 3B). The cortical layer develops a series of infoldings (Fig. 3C) that intrude into the inner layer on the medulla. As these infolds become more pronounced, clefts (CF) form and medulla begins to deliquesce, transforming into the volva-gel (VG) within the cortex (Fig. 3D). The primordium increases in size throughout this phase as the cortex and other internal structures develop to form recognizable small myco-eggs.

Myco-Egg Phase: The clefts further deepen and become compressed, forming peridial sutures (PS) at the myco-egg centres. The deepest point of each peridial suture differentiates into palisade tissue (PT) (Fig. 3E) that comprises the gleba fundamentals. At the peridial suture-palisade tissue junction, an intermediate tissue (IT) develops (Fig. 3F) and then thickens into hyphal knots (HK) while the palisade transforms into pseudoparenchymatous tissue (PP) (Fig. 3G). The hyphal knot begins to divide, branching out on three sides to initiate the receptacle (IR). Each pseudoparenchymatous mass further differentiates to form a glebal initial (GI) (Fig. 3H). The lowermost peridial suture ring producing lower branches proliferates towards the base, each fusing together to form a very short stipe (ST). The growing receptacle (GR) develops further

Fig. 2. *Clathrus delicatus* (KUABSAK-MCH 265). —A White mycelial strands. —B Mycelium covering twigs of *Bambusa arundinacea*. —C A cluster of myco-eggs. —D Mucilaginous substance (volva-gel) coating the emerging receptacle. —E The latticed network of the mature receptacle. —F Arms at the basal portion of the receptacle united to form a short stipe. —G Glebifer. —H Basidiospores. Magnifications: A–C = 15×, D = 40×, E = 12×, F = 35×, G = 45×; scale bar: H = 5 µm.



Figs. 3A-J. Sporoma development of *Clathrus delicatus* (KUABSAK-MCH 265).

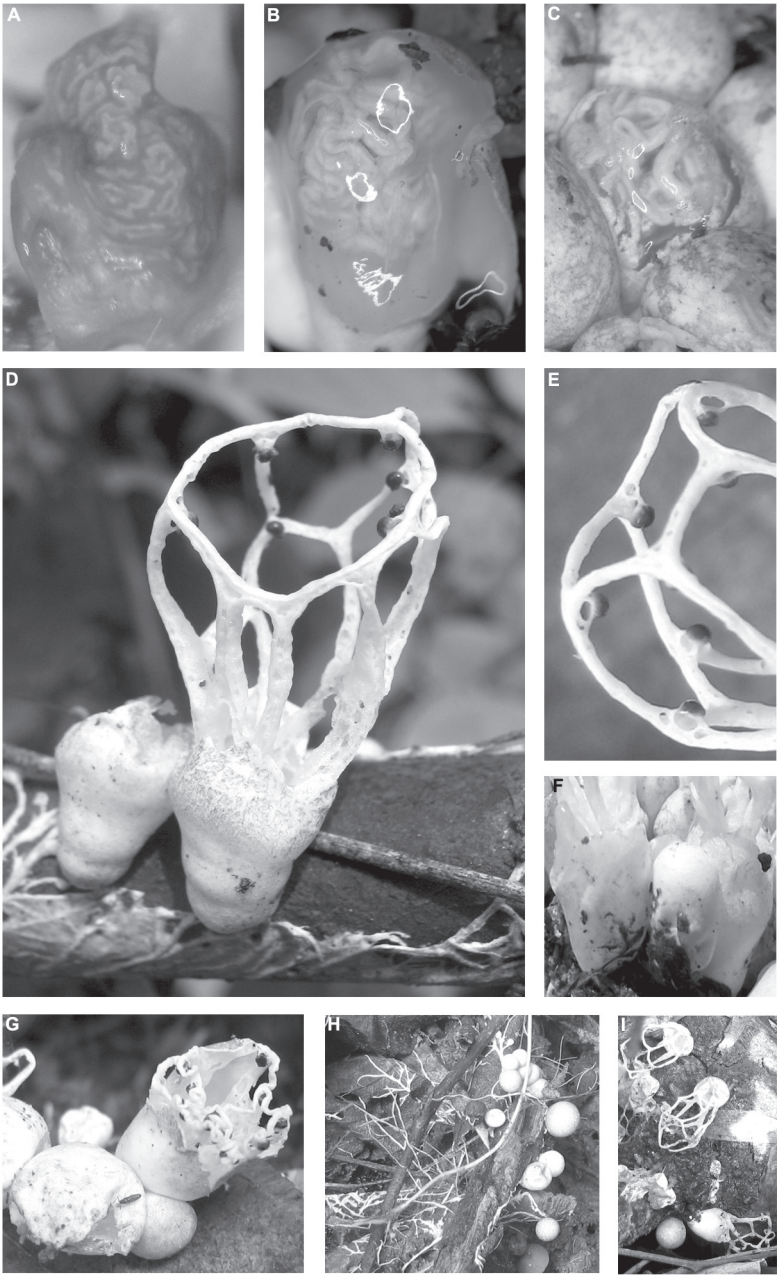
Abbreviations: C—Cortex, CF—Cleft, CT—Palisade tissue transforming into columella and trama, GR—Growing receptacle, HK—Hyphal knot, GI—Gleba initial, IR—Initiation of receptacle, IT—Intermediate tissue, M—Medulla, P—Primordium, PP—Pseudoparenchyma layer, PS—Peridial suture, PT—Palisade tissue, RG—Reduction of glebal mass, ST—Stipe, VG—Volvagel.

with the reduction of glebal mass (RG) (Fig. 3I). The continuous development and branching of the receptacle (R) at the centre displaces the volva-gel towards the periphery as the peridial sutures degrade (Fig. 3J) and the central medulla disintegrates. The palisade tissue completely transforms into gelatinized columella and trama (CT), which adheres tightly to the developing receptacle that completely surrounds it (Fig. 4A). After 8–10 days, the volva-gel becomes more viscous (Fig. 4B) as the egg increases in size and basidiospores are formed from hymenial layers forming inverted cup shaped structures (gleba) at the junction of the arms. The mature egg has three distinct layers: the exoperidium (outer skin), mesoperidium (volva-gel), and endoperidium (receptacle and gleba). After the egg ruptures apically (Fig. 4C), the expanding receptacle emerges.

Receptacle phase: Rupture is caused by increasing turgor pressure and cell elongation in the expanding receptacle. The receptacle freely expands and this phase proceeds rapidly (2–4 minutes) until the mature sporoma has formed (Fig. 4D), with the gleba found at the arm intersections resembling three-legged stools (Fig. 4E). After 'hatching,' the ruptured exoperidium remains behind as a volva (Fig. 4F) attached to the mycelial strands. The receptacle eventually shrinks with time (Fig. 4G), and insects attracted by the fetid glebal odor disseminate the spores, thus continuing the life cycle with multiple colonies (Fig. 4H) and developing sporomata (Fig. 4I).

Discussion

In *Clathrus*, receptacle morphology varies considerably, as does the placement of the gleba within the receptacle. *Clathrus archeri* (Berk.) Dring, *C. crispatus* Thwaites ex E. Fisch., *C. kusanoi* (Kobayasi) Dring, *C. mauritianus* (Lloyd) Dring, and *C. ruber* P. Micheli ex Pers. have gleba distributed over a large portion (with the exception of the more basal areas) of the inner surfaces of the receptacle (Dring 1980, Arora & Burk 1982). In *C. baumii* Henn. and *C. preussii* Henn., the gleba spreads over the inner arm surfaces of the arms but tends to concentrate near where the arms intersect. In *C. columnatus* Bosc the gleba is found only at the more apical portions of the receptacle as a centralized glebal mass that spreads down along the inner surface of the arms (Dring 1980). In *C. chrysomycelinus* and *C. oahuensis* Dring the gleba is restricted to discrete droplets in glebifers seated on the intersection of the arms (Dring et al. 1971, Dring 1980). Finally, although the gleba of *C. delicatus* is also restricted to the arm intersections, the droplets are very minute, and the glebifers are even more specialized in their structure, resembling miniature three-legged stools (Dring 1980).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Dring et al. (1971) suggested that variation found within *Clathrus* could be interpreted in an evolutionary context, which Dring (1980) later placed in several evolutionary "series." From the "primitive" state, generally these series progressively "simplified" in the distribution of the gleba, accompanied by a reduction in glebal quantity. These trends were also associated with a reduced receptacle size as well as with an increasing complexity in the localization of the gleba, with glebifers occurring in the most advanced forms (Dring 1980). *Clathrus delicatus* was considered one of the more advanced species in *Clathrus*, exhibiting the most specialized and complex glebifer form (Dring et al. 1971, Dring 1980). Here we also document for *C. delicatus* the extremely small receptacle size (15–20 × 10–14 mm), which Dring (1980) also considered a more evolutionarily advanced trait.

A recent molecular phylogeny of the *Phallomycetidae* (Hosaka et al. 2006) included *Clathrus ruber* and *C. chrysomycelinus*, as well as other species in the *Phallales*. Although many early authors (Fischer 1898–99, Lloyd 1906, Petch 1908) suggested that *Clathrus* is the most primitive genus within the *Clathraceae*, Hosaka et al. (2006) placed *Clathrus* species within a more recently derived *Clathraceae* clade that is sister to the *Phallaceae* clade. As Hosaka et al. (2006) only included two species of *Clathrus* in their study, evolutionary relationships among *Clathrus* species remain poorly understood.

Acknowledgements

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Fig. 4. Sporoma development of *Clathrus delicatus* (KUABSAK-MCH 265). — A Gelatinized tissue adhering to the developing receptacle. —B Volva-gel enveloping the receptacle. —C Apical rupturing of the myco-egg. —D Expanded receptacle. —E Gleba at the intersections of arms. —F Volva. —G An aged receptacle, shrinking with desiccation. —H Mycelium strands with eggs forming intermittently. —I Expanded receptacles of a colony.

Magnifications: A = 60×, B–C, E = 25×, D, F = 20×, G = 10×.

Literature cited

Alexopoulos CJ, Mims CW, Blackwell M. 2002. Introductory mycology. 4th ed. John Wiley & Sons, Singapore. 869 pp.

Apte D. 2005. First record of *Clathrus delicatus* Berkeley & Broome (1873) from Sanjay Gandhi National Park. Journal of Bombay Natural History Society 102: 135–136.

Arora D, Burk WR. 1982. *Clathrus archeri*, a stinkhorn new to North America. Mycologia 74: 501–525. doi:10.2307/3792972

Beltrán-Tejera E, Bañares-Baudet A, Rodríguez-Armas JI. 1998. Gasteromycetes of the Canary Islands: some noteworthy new records. Mycotaxon 68: 439–453.

Berkeley MJ, Broome CE. 1873 ["1875"]. Enumeration of the fungi of Ceylon. Part II., containing the reminder of the hymenomycetes, with the remaining established tribes of fungi. Journal of the Linnean Society, Botany 14: 29–140.

Bessey ER. 1950. Morphology and taxonomy of fungi. Philadelphia, Blakiston 791 pp.

Cunningham GH. 1944. The gasteromycetes of Australia and New Zealand. John McIndoe, New Zealand. 236 pp.

Dring DM. 1980. Contributions towards a rational arrangement of the *Clathraceae*. Kew Bulletin 35: 1–96. doi:10.2307/4117008

Dring DM, Meeker J, Goos R. 1971. *Clathrus oahuensis*, a new species from Hawaii. Mycologia 63: 893–897. doi:10.2307/3758056

Fischer ED. 1898-99 ["1900"]. *Phallineae*. 276–296, in: Engler A, Prantl K (eds), Die Natürlichen Pflanzenfamilien, vol. 1(1**). Wilhelm Engelmann, Leipzig.

Gäumann EA. 1952. The fungi. Hafner publishing Co., London. 420 pp.

Hosaka K, Bates ST, Beever RE, Castellano MA, Colgan III W, Domínguez LS, Nouhra ER, Geml J, Giachini AJ, Kenney SR, Simpson NB, Spatafora JW, Trappe JM. 2006. Molecular phylogenetics of the gomphoid–phalloid fungi with an establishment of the new subclass *Phallomycetidae* and two new orders. Mycologia 98: 949–959. doi:10.3852/mycologia.98.6.949

Ingold CT. 1971. Fungal spores. Clarendon Press, Oxford. 153 pp.

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Ainsworth and Bisby's dictionary of the fungi, 10th ed. CABI Publishing, UK. 771 pp.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour, 3rd ed. Eyre Methuen, London. 252 pp.

List PH, Freund B. 1968. Geruchstoffe der stinkmorchen, *Phallus impudicus* L. Miteilung uber Pilzinhaltstoffe. Planta Medica supplement 18: 123–132. doi:10.1055/s-0028-1099948

Lloyd CG. 1906. Concerning the phalloids. Mycological Writings 2: 293–308.

Narasimhan MJ. 1932. The *Phalloideae* of Mysore. Journal of Indian Botanical Society 11: 248–254.

Pegler DN, Læssøe T, Spooner BM. 1995. British Puffballs, Earthstars and Stinkhorns. Royal Botanic Gardens, Kew. 265 pp.

Petch T. 1908. The *Phalloidae* of Ceylon. Annals Royal Botanical Garden Peradeniya 4(4): 60–165.

Smith AH, Schaffer RL. 1962. Key to the selected groups of the higher fungi. University of Michigan, London. 264 pp.